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Cardiac dysfunction in rats prone to audiogenic epileptic seizures

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ABSTRACT

Purpose: Cardiac dysfunction is one of the possible causes of sudden unexpected death in epilepsy (SUDEP). Therefore, the objective of this study was to evaluate cardiac and electrocardiographic parameters in rats with audiogenic epileptic seizures (WAR – Wistar audiogenic rats).
Methods: *In vivo* arterial pressure, heart rate (HR), autonomic tone and electrocardiography (ECG) were measured in awake animals in order to examine cardiac function and rhythm. *Ex vivo*, the Langendorff technique was used to analyze the cardiac function and the severity of reperfusion arrhythmias. *In vitro*, confocal microscopy was used to evaluate calcium transient parameters of isolated ventricular cardiomyocytes.
Results: *In vivo* autonomic tone evaluation revealed enhanced sympathetic activity, changes in cardiac function with increased systolic arterial pressure and higher basal HR in WAR. In addition, ECG analysis demonstrated electrical alterations with prolongation of the QT interval and QRS complex in these animals. *Ex vivo*, we observed a decrease in systolic tone and HR and an increase in the duration of ischemia/reperfusion arrhythmias in WAR. Moreover, intracellular Ca^{2+} handling analysis revealed an increase in the peak of calcium and calcium transient decay in audiogenic rats. Treatment with atenolol (β1-adrenergic antagonist) normalized the systolic tone, reduced cardiac hypertrophy and the associated increase in the susceptibility to reperfusion arrhythmias observed in WAR.
Conclusion: We present evidence that chronic disturbances in sympathetic tone in WAR cause increases the risk to life-threatening arrhythmias. Our results support a relationship between seizures, cardiac dysfunction and cardiac arrhythmias, which may contribute to the occurrence of SUDEP.

1. Introduction

The mortality among patients with epilepsy is on average 2–3 times greater than that in the general population.[1] The commonest cause of death in young adults with epilepsy is sudden unexpected death in epilepsy (SUDEP). The underlying mechanisms that cause SUDEP are still unclear. Several different mechanisms may be involved, and there may be no single explanation for all cases. For example, one mechanism, which has been suggested, involves cerebrogenic cardiac arrhythmia and autonomic dysfunction.[2] There are limited observations of SUDEP with case reports of observed cardiac arrhythmias.[3] It has been suggested that SUDEP may be caused by dysfunction of the cardiovascular autonomic system, which exposes the patient to arrhythmias and sinus arrest.[4]

The development of cardiovascular autonomic dysfunction during the interictal period has been shown to be associated with the epileptogenic activity.[1] Experimental data suggest that interictal epileptogenic activity induces autonomic imbalance, which may be associated with cardiac arrhythmias.[5] Autonomic symptoms frequently occur during epileptic seizures either as an accompaniment to other seizure symptoms or as the predominant seizure manifestation.[6]

Cardiac rhythm and conduction abnormalities are common during seizures, particularly if the seizure is prolonged or generalized.[7] Electrocardiogram (ECG) abnormalities with potentially serious changes including ST-depression and T-wave inversion were observed in patients during the ictal and postictal period, suggesting that myocardial ischemia associated with ictal sympathetic storms may lead to lethal arrhythmias.[7,8] Natelson et al.[9] observed that patients with epilepsy, who died suddenly and

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unexpectedly presented cardiac pathological conditions that could be responsible for their deaths. In addition, Tigaran et al.[10] suggested secondary cardiac damages in epileptic patients since they found signs of ischemia on ECG and elevated cardiac enzymes (troponin). Alehan et al.[11] showed presence of elevated brain natriuretic peptide (BNP) and creatine kinase (CK-MB) in patients with seizures, the first evidence of subtle cardiac dysfunction in epilepsy patients.

In a previous study,[12] we observed that convulsive seizures triggered by maximal electroshock (MES) induced profound abnormalities in cardiac rhythm and increased the incidence/duration of ischemia-reperfusion arrhythmias in Wistar rats. Furthermore, Metcalf et al.[13] suggested that status epilepticus produces tachycardic ischemia, following the activation of the sympathetic nervous system in rats, resulting in cardiac myofilament damage, arrhythmogenic alterations in cardiac electrical activity, and increased susceptibility to ventricular arrhythmias. Fazan Jr et al.[14] observed that autonomic imbalance (sympathetic predominance) in Wistar audiogenic rats (WAR) might be associated with an increased risk of life-threatening cardiovascular events in this strain. Therefore, taking into consideration the disruption of the normal autonomic control and cardiac alterations suggestive of myocardial injury during epileptic seizures, the aim of this study was to evaluate the cardiovascular and electrocardiographic parameters of rats predisposed to seizures induced by audiogenic stimulus.

2. Methods

2.1. Animals

Experiments were performed in male Wistar rats (250–300 g, $n = 22$) from the main breeding stock of the Institute of Biological Sciences (Federal University of Minas Gerais, Brazil) and WAR (250–300 g, $n = 46$) from the inbred colony maintained at the Department of Physiology and Biophysics (Institute of Biological Sciences, Federal University of Minas Gerais, Brazil). The WAR strain is a genetic model of sound-induced reflex epilepsy that, in the acute situation, mimics tonic-clonic seizures (audiogenic seizures). All animals were housed individually in plastic cages, under controlled lighting conditions (lights on at 6:00 am and off at 8:00 pm), room temperature at 24 °C and with food and water *ad libitum*. All efforts were made to avoid any unnecessary distress to the animals and all animal procedures were performed in accordance with institutional guidelines approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais, Brazil (CETEA-UFMG), which are in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.2. Acoustic stimulation and behavioral evaluation of seizure severity

The apparatus used to induce acoustic stimulation consisted of a cylindrical transparent cage inside a larger, sound-proof box, provided with a door and frontal glass window for observation. A sound stimulus (120 dB SPL) was delivered into the acoustic chamber through a loudspeaker until tonic seizures appeared or during a maximum period of 1 min. Behavior was assessed by direct observation using a set of discrete behavioral categories and quantified by means of a severity index (SI) scale ranging from zero to one.[15] All animals were submitted to a screening procedure (at 70, 74, and 78 days of age) in order to determine seizure severity and to evaluate audiogenic susceptibility. The WAR display epileptic behavior after sound stimulation, presenting running fits, jumping and atonic falling, followed by tonic-clonic seizures and clonic spasms. The tests were always conducted after 4:00 pm and all seizures were induced since these animals do not have spontaneous seizures.[16] A resting period of at least one week after screening was allowed before the initiation of the experimental protocols. The most frequent behavioral sequences produce the following SI values: 0.11 = wild running with only one running fit; 0.23 = wild running with only one running fit, jumping and atonic falling; 0.38 = wild running with two running fits, jumping and atonic falling; 0.61 = all of the above plus tonic convulsion (back arching tonus); 0.73 = all the above plus partial (only forelimb or hindlimb) and generalized (forelimb and hindlimb) clonic seizures; 0.85 = all the above plus clonic spasms; 0.90 = all the above plus ventral flexion of the head; 0.95 = all the above plus forelimb hyperextension; 1.0 = all the above plus forelimb and hindlimb hyperextension.[17] In the present study only animals with SI > 0.85 (running, jumping plus clonic spasms) were included in the experimental groups.

2.3. Arterial pressure measurements

Five Wistar rats and five WAR were used for recording arterial pressure and heart rate (HR). Twenty-four hours before the experiments, the animals were anesthetized with 2.5% tribromoethanol (1 mL/100 g of body weight, i.p., Sigma–Aldrich, Inc.) and polyethylene catheters (PE-10 connected to PE-50) were inserted into abdominal aorta through the left femoral artery and into the femoral vein for recording arterial pressure and drug infusion, respectively. The catheters were tunneled subcutaneously and exteriorized backing the cervical region of the animal. The hemodynamic parameters: pulse arterial pressure (PAP), mean arterial pressure (MAP) and systolic and diastolic arterial pressure (SAP and DAP, respectively) were monitored simultaneously during experiments by a solid-state strain gauge transducer (TSD 104A, Biopac Systems, Inc., CA, USA). The HR was determined from the SAP. The transducer was connected to a computer through a data acquisition system (MP100; Biopac Systems, Inc., CA, USA). The data were analyzed by the AcqKnowledge Software. The experiments were conducted in conscious and freely moving rats.

2.4. Vagal and sympathetic activity evaluation

After the measurement of the arterial pressure and HR, the vagal and sympathetic activities were assessed in the same animals, *i.e.* five Wistar rats and five WAR. Before drug administration, HR and MAP were monitored during 20 min (baseline period) in conscious freely moving rats. After stabilization, vagal and sympathetic activities were assessed by intravenous injections of methylatropine (MA; 3 mg/kg; Sigma–Aldrich, Inc.) or atenolol (4 mg/kg; Sigma–Aldrich, Inc.) at a maximal volume of 0.2 mL per injection. On the first day, MA was injected after recording of the resting HR. Because the HR response to MA reached the peak within 10–15 min, this time interval was standardized for measurement of HR. Atenolol was injected 15 min after MA injection and the responses were measured after 10–15 min. To obtain the reverse sequence of the blockade, atenolol was administered before the application of MA in the second day of experiment. The efficacy of the blockade induced by MA and atenolol was confirmed by the elimination of the reflex changes in HR produced by phenylephrine (8 μg/kg; Sigma–Aldrich, Inc.) and sodium nitroprusside (50 μg/kg; Sigma–Aldrich, Inc.) administration, respectively, at a maximal volume of 0.2 mL per injection. The autonomic tone was evaluated as previously described.[18] A diagram showing the protocol used to determine the cardiac sympathetic and parasympathetic tone and effects is presented in Fig. 1. At the end of the protocol, the wet weight of the hearts was recorded, normalized by the tibia length and then expressed as mass index (mg/mm).